PUBLICATIONS AND PATENTS WITH ABSTRACTS

ENZYME RESEARCH DIVISION
Bureau of Agricultural and Industrial Chemistry
Agricultural Research Administration
United States Department of Agriculture

Western Regional Research Laboratory
Albany, California

January, 1949 - July, 1950

A limited number of reprints of publications are available. Those not available are marked with an asterick (*).

Journal Articles:

E. F. Jansen, M.-D. F. Nutting, R. Jang and A. K. Balls. Inhibition of the proteinase and esterase activities of trypsin and chymotrypsin by diisopropyl fluorophosphate: Crystallization of inhibited chymotrypsin. Jour. Biol. Chem., 179:189-199. May, 1949.
Diisopropyl fluorophosphate (DFP) was found to be without effect on chymotrypsinogen. Trypsin was inhibited by minute amounts of DFP, the inhibition being greater at neutrality than at acid pH values. The inhibition reaction was progressive with time. The esterase and proteinase activities of trypsin were equally affected, showing that the two activities probably reside in the same active centers of the molecule. Chymotrypsin is inhibited by DFP. At pH 7.5, 50 per cent inhibition occurred when the molar ratio of inhibitor to enzyme was 0.55:1. Approximately one mole of inhibitor per mole of enzyme was required for complete inhibition. The completelyinhibited chymotrypsin was crystallized thereafter by the procedure used for the active enzyme. After a recrystallization, dialysis, and lyophilization, the enzyme was still completely inhibited.

E. F. Jansen, M.-D. F. Nutting, and A. K. Balls. Mode of inhibition of chymotrypsin by diisopropyl fluorophosphate. I. Introduction of phosphorus. Jour. of Biol. Chem., 179:201-204. May, 1949.
By the use of radioactive DFP it was found that the phosphorus of DFP was introduced into crystalline chymotrypsin by the inhibition reaction of DFP on chymotrypsin. The amount of phosphorus introduced was 1.1 moles per mole of enzyme. This amount of phosphorus corresponds to the amount of DFP previously found necessary to inhibit chymotrypsin completely. Chymotrypsinogen under the same conditions did not react with DFP. Hence the conversion of the zymogen to the active enzyme liberates not only the groups responsible for activity but also those with which DFP reacts, the two possibly being the same. It is suggested that the mode of action of DFP may well differ from other phosphate ester inhibitors.

O. H. Emerson. The bitter principle in Navel oranges. Food Tech., 3:248. July, 1949.
Limonin was found as the sole isolable bitter substance in Navel orange juice. Aside from its bitterness, it appears inert physiologically. Limonin was also obtained from grapefruit seeds, and with a new bitter substance, nomilin, $C_{28}H_{34}O_9$, from the seeds of Valencia oranges and lemons. Like limonin, nomilin contains a carbonyl and is a dilactone, but alkali splits out acetic acid, and on acidification there is formed a lactone-acid $C_{26}H_{32}O_8$.

S. Schwimmer, and A. K. Balls. Isolation and properties of crystalline alpha-amylase from germinated barley. Jour. Biol. Chem., 179:1063. 1949.
Isolation and crystallization of the alpha-amylase of germinated barley (malt) has been described in detail. The procedure consists essentially of heating concentrated malt extract, precipitating the remaining protein with ammonium sulfate, adsorption of the enzyme from an alcoholic solution on wheat starch granules, and crystallization of the eluted enzyme from ammonium sulfate. Behavior of the fractions leading to crystallization and some of the properties of the crystalline substance, including molecular weight, purity, and ultraviolet absorption spectrum, are reported. The kinetics of hydrolysis, the requirement of the enzyme for calcium, and the apparent absence of inositol in the enzyme are also discussed.

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The adsorption of malt alpha-amylase in 40 per cent ethanol at 0° on granules, modifications, and fractions of starch has been studied. The efficiency of adsorption is inversely proportional to the granule size, or, proportional to the surface area of the granules. Various kinds of treatment of the granule increase this efficiency somewhat. The adsorption obeys, within limits, the Freundlich adsorption isotherm. Starch fractions (i.e., amylopectin, dextrins, amylose, glycogen) exhibit a different higher order of magnitude of adsorption than do the granules. The exact degree of efficiency depends upon the structure, the source, and fineness of subdivision of the substance used. The degree of adsorption is independent of the state of purity of the enzyme. Maltose and limit dextrin decrease, whereas alpha dextrin increases the extent of adsorption.

W. G. Rose. A micromethod for detecting the enzymatic breakdown of cephalins; and/or phosphoserines. Food Tech., 4:230-232. June, 1950.
A diffusion procedure is described in which enzymatic digests are oxidized with periodate in a Petri dish, and the ammonia so formed is diffused into sulfuric acid spread on the ground surface of the glass plate cover of the Petri dish. The diffused ammonia is estimated by nesslerization. The adequacy of the method was demonstrated with standard ethanolamine solutions and also with an alkaline hydrolyzate of synthetic palmitolinoleocephalin. As the method measures only those free bases that form ammonia with periodate, it is specific for the liberation of ethanolamine or serine, and so distinguishes phosphatidyl ethanolamines and phosphatidyl serines from the lecithins. The method clearly demonstrated the

existence of an enzyme system in cabbage juice, capable of liberating ethanolamine or serine from a phospholipide, but unable to split synthetic cephalin.

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E. F. Jansen, M.-D. F. Nutting, R. Jang, and A. K. Balls. Mode of inhibition of chymotrypsin by DFP. II. Introduction of isopropyl and elimination of fluorine as hydrogen fluoride. Jour. Biol. Chem., 185:209. July, 1950.
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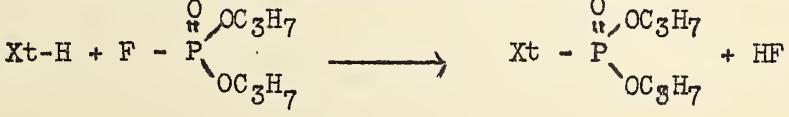

where Xt-H represents chymotrypsin having some particular, active hydrogen.

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